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Title of the invention

Treatment of insufficient perfusion

Field of the invention

The present invention relates to methods in the treatment, prevention and/or alleviation of insufficient perfusion in an organ or organs, regardless of underlying causes, as well as compositions for said purposes. The invention in particular relates to the treatment, prevention and/or alleviation of insufficient perfusion as a result of embolism, in particular pulmonary thromboembolism.

Background of the invention

Embolism

An embolus is a foreign object, a quantity of air or gas, a fat globule, a bit of tissue or tumour, or a piece of a thrombus that circulates in the bloodstream until it becomes lodged in a vessel, partially or completely obstructing blood flow.

Pulmonary embolism is a common disorder accompanied by a significant morbidity and mortality. Thromboembolism may either be acute through activation of the blood clotting system and disseminated intravascular coagulation, or occur at a later stage through the formation of thrombi in the pulmonary vessels or formation in the venous circulation with subsequent embolisation to the lung. The cause behind thrombi in the lung can also be so called thrombotisation, i.e. the formation of microthrombi in the circulation, triggered by tissue factors in the blood vessels. These microthrombi travel in the circulation until becoming trapped in the capillaries in the lung. It is estimated that up to 40 % of all cases of pulmonary embolism may be of this origin.

It is also estimated that pulmonary embolism is the main or at least a contributory cause of in-patient death. Swedish autopsy records indicate that pulmonary embolism is involved in about 20 % of in-patient deaths. Pregnant women, and in

particular women undergoing caesarean section; cancer patients; trauma victims, and patients undergoing surgery, e.g. orthopaedic surgery, are at risk. Further risk groups include, but are not limited to, individuals confined to bed rest or other types of confinement or restriction in the movement of the body of limbs, both during medical treatment or recovery from such treatment, or during transportation, e.g. air travel. Still further risk groups include, but are not limited to patients with infections, suffering from diseases or undergoing pharmaceutical treatments disturbing the blood clotting system or the system for resolution of blood clots.

One special form of pulmonary embolism, pulmonary gas embolism, is a well-known consequence of surgery, trauma, diving and aviation, including the exploration of space. Another form, pulmonary thromboembolism, is caused when a thrombus or fat globule travels in the blood to the lungs as a result of trauma, surgery or dislodging of a thrombus or part thereof from another location in the body, e.g. in deep venous thrombosis (DVT).

In the majority of the cases of pulmonary embolism, the source is deep venous trombosis (DVT). Venous tromboembolic disease is the third most common cardiovascular disease after ischemic coronary heart disease and stroke. The most common treatments of pulmonary embolism include the administration of nasal oxygen, infusion of anticoagulantia and/or trombolytica, and surgical intervention. In the administration of thrombolytica, bleeding is a serious complication, which has to be considered. Inhaled nitric oxide (NO) has been tried experimentally, but consensus has not been reached if such treatment is efficacious or not.

The pathophysiology of pulmonary embolism is pulmonary macro- or micro-obstruction, depending on emboli size, leading to pulmonary hypertension of varying severity. Acute pulmonary hypertension may cause right ventricle failure (acute cor pulmonale) and eventually cardiogenic chock. Treatment of acute pulmonary hypertension must therefore include reduction of pulmonary afterload, preferably pulmonary vasodilators with no systemic effects. Another feature of

pulmonary embolism is disturbances of blood gases of varying degree, indicating ventilation-perfusion matching failure, though normal or disturbed blood gases are not conclusive for pulmonary embolism. For example, PaO_2 is likely to be decreased after acute massive pulmonary embolism but may be normal in patients with sub-massive pulmonary embolism.

For more information on pulmonary embolism, see "Guidelines on diagnosis and management of acute pulmonary embolism", Task Force on Pulmonary Embolism, European Society of Cardiology, European Heart Journal 2000;21:1301-1336.

Insufficient perfusion also occurs in other instances, such as transplantation and in individuals confined to bed rest or other types of confinement or restriction in the movement of the body of limbs, both during medical treatment or recovery from such treatment, or during transportation, e.g. air travel.

Nitric oxide

Nitric oxide (NO) is a molecule of importance in several biological systems, and is continuously produced in the lung and can be measured in ppb (parts per billion) in expired gas. The discovery of endogenous NO in exhaled air, and its use as a diagnostic marker of inflammation dates back to the early 1990-ies (See e.g. WO 93/05709; WO 95/02181). Today, the significance of endogenous NO is widely recognised, and since a few years back, a clinical analyser is available on the market (NIOX®, the first tailor-made NO analyser for routine clinical use with asthma patients, AEROCRINE AB, Solna, Sweden).

In the summer of 1997 the European Respiratory Journal published guidelines (ERS Task Force Report 10:1683-1693) for the standardisation of NO measurements in order to allow their rapid introduction into clinical practice. Also the American Thoracic Society (ATS) has published guidelines for clinical NO measurements (American Thoracic Society, Medical Section of the American Lung Association: Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric

oxide in adults and children – 1999, in Am J Respir Crit Care Med, 1999; 160:2104-2117).

In early experiments attempting to elucidate the role of NO in respiratory gas, massive helium or air emboli were used to stop the circulation in the lungs of test animals. The results indicated that increased levels of NO could be detected in exhaled air (Gustafsson *et al.*, Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Commun* 1991;181:852-7).

In 1999, Deem *et al.* published results indicating that hemodilution during venous gas embolisation improves gas exchange, without altering V(A)/Q or pulmonary blood flow distributions (Anesthesiology., 1999 Dec;91(6):1861-72). A continuous infusion of nitrogen through the left internal jugular vein at a rate of approximately 0.006 ml/kg min was applied to achieve embolisation. In the results, an increase of NO is recorded in embolised, hemodiluted anaemic test animals, but not in undiluted controls. The authors state that the difference between baseline and T1 VNO was statistically significant only for anaemic animals. In practice, no increase in NO was recorded for animals with normal hematocrit. Deem *et al.* also discuss the limitations of the model, venous gas embolisation using a continuous infusion of small bubbles, and states that it may be dissimilar to cases of air embolisation in the clinical setting, and that extrapolation of the data to clinical management is difficult.

The effect of vasodilator therapy was investigated in a canine model of acute pulmonary hypertension (Priebe, Am. J. Physiol. 255 (Heart Circ. Physiol, 24):H1232-H1239, 1998). In this study, pulmonary embolisation was simulated by injecting a suspension of finely chopped muscle tissue in saline containing 2000 U of heparin. Small volumes (0.5 – 2 ml) of the muscle suspension was injected repeatedly through a femoral vein catheter until the mean pulmonary arterial pressure had increased approximately threefold.

It is generally recognised that endogenous generation of the gaseous molecule nitric oxide (NO) plays an important role in the modulation of pulmonary vascular

tone to optimise ventilation-perfusion matching (Persson *et al.* 1990). In healthy human adults, NO is of importance in regulation of both basal pulmonary and systemic vascular resistance (Stamler *et al.* 1994). Local regulation of blood flow is influenced by administration of NO synthase inhibitor in healthy human subjects (Rimeika *et al* 2004). Vasodilator effects of endogenous NO in the postnatal pulmonary circulation clearly contribute to the adaptations of the fetal lung to air breathing at delivery (Abman *et al.* 1990). NO generation in the postnatal lung is stimulated for example by mechanical stretch, increased shear forces and increased O_2 tension in the alveoli (Heymann 1999). Measuring NO in exhaled breath is a good way of monitoring changes in endogenous NO production or scavenging in the lung (Gustafsson *et al.* 1991).

Since ventilation-perfusion matching disturbances and increased pulmonary artery blood pressure are features of pulmonary embolism, inhaled NO has been tested as treatment. Nevertheless treating pulmonary embolism with inhaled NO has yielded conflicting results e.g. improvement of hemodynamics but no improvement of blood gases (Tanus-Santos JE & Theodorakis MJ, 2002).

Therefore and with knowledge of the importance of NO in the pulmonary circulation, the present inventors set out to examine: 1) the response of intact endogenous NO generation and 2) and the significance of this response by inhibiting endogenous NO production in an experimental rabbit model of pulmonary embolisation.

One objective behind the present invention was to identify new methods and compositions for the treatment, alleviation and/or prevention of insufficient perfusion, in particular pulmonary embolism. Another objective was to identify a method and composition which is easy and safe to administer, and which does not exhibit the side effects of conventional treatments and drugs. Other objectives, the solutions reached and the advantages associated therewith will become evident upon study of the description and examples.

Summary of the invention

The present inventors surprisingly found that a compound, capable of delivering nitric oxide (NO), generating NO or increasing the endogenous production of NO can be advantageously used to increase perfusion in an organ, exemplified by the lung in a model of pulmonary embolism. Consequently, the inventors make available methods and compositions as defined in the attached claims, incorporated herein by reference.

Short summary of the drawings

The invention will be described in closer detail in the following description, examples, and attached drawings, in which

Fig. 1 shows the changes in mixed exhaled nitric oxide (FE_{NO}) upon muscle emboli challenge (time 0) in artificially-ventilated pentobarbital anaesthetised rabbits. In group 1 (n=6, open circles and bar, 58 mg kg^{-1}) and in group 2 with inhibited NO-production (n=4, L-NAME 30 mg kg^{-1}, closed circles and bars, 7.5 mg kg^{-1}). The bars show the infusion times. * indicates $p<0.05$ compared to control levels (time= -5)

Fig. 2 shows changes in end-tidal CO_2 ($ETCO_2$) upon muscle emboli challenge (time 0) in artificially-ventilated pentobarbital anaesthetised rabbits. Group 1 (n=6, open circles and bar, 58 mg kg^{-1}) and in group 2 with inhibited NO-production (n=4, L-NAME 30 mg kg^{-1}, closed circles and bars, 7.5 mg kg^{-1}). The bars show the infusion times. "3/4" and "2/4" indicates survival in group 2 (L-NAME group) at the indicated time points.

Fig. 3 shows changes in mean arterial blood pressure (MAP) upon muscle emboli challenge (ME, time 0) in artificially-ventilated pentobarbital anaesthetised rabbits. Group 1 (n=6, open circles and bar, 58 mg kg^{-1}) and in group 2 with inhibited NO-production (n=4, L-NAME 30 mg kg^{-1}, closed circles and bars, 7.5

mg kg^{-1}). The horizontal bars labelled ME show the infusion times. "3/4" and "2/4" indicates survival in group 2 (L-NAME group) at the indicated time points.

Fig. 4 shows changes in heart rate (HR) upon muscle emboli challenge (time 0) in artificially-ventilated pentobarbital anaesthetised rabbits. Group 1 (n=6, open circles and bar, 58 mg kg^{-1}) and in group 2 with inhibited NO-production (n=4, L-NAME 30 mg kg^{-1}, closed circles and bars, 7.5 mg kg^{-1}). The horizontal bars labelled ME show the infusion times for the muscle embolism challenge. "3/4" and "2/4" indicates survival in group 2 (L-NAME group) at the indicated time points.

Fig. 5 and 6 show arterial oxygen tension and haemoglobin oxygen saturation in rabbits challenged with Muscle embolus (unpretreated, 58 mg kg-1; n=6) as indicated by horizontal bar labelled ME.

Fig. 7 and 8 show arterial carbon dioxide tension and pH in rabbits challenged with muscle embolus (unpretreated, 58 mg kg-1; n=6) as indicated by horizontal bar labelled ME.

Fig. 9 shows percent survival in unpretreated control animals (artificially-ventilated pentobarbital anaesthetised rabbits) (open circle, n=6 and 2 for 58 and 120 mg kg^{-1} respectively) receiving muscle embolus challenge (ME) at 58 to 120 mg kg^{-1}, and in animals receiving 7.5 to 30 mg kg^{-1} muscle embolus challenge after pre-treatment with L-NAME (30 mg kg^{-1}; filled circle, n=4, 2 and 2 for 7.5, 15 and 30 mg kg-1 respectively).

Fig. 10 is a graph showing changes in mixed exhaled nitric oxide (FE_{NO}) in artificially-ventilated pentobarbital anaesthetised rabbits upon infusions of NO-gas dissolved in either saline or lipid emulsion in an animal with inhibited NO-production (L-NAME 30 mg kg^{-1}, closed circles and bars. The bars show the infusion times.

Fig. 11 is a graph showing changes in metHb in arterial blood in artificially-ventilated pentobarbital anaesthetised rabbits upon infusions of NO-gas dissolved in either saline or lipid emulsion in an animal with inhibited NO-

production (L-NAME 30 mg kg^{-1}, closed circles and bars. The bars show the infusion times.

Description

Experiments performed by the present inventors indicate that the administration of inhaled NO is not a sufficient treatment in cases of pulmonary embolism. Experiments using gaseous NO dissolved in physilogical saline, given as an injection of up to 5 ml per kg body weight have been performed. Surprisningly, no increase of exhaled NO could be detected. Similarly, no changes in blood circulation were to be seen. This indicates that NO is rapidly decomposed or otherwise inactivated in saline, or that it does not reach the lungs or the systemic vessels.

Therefore it was highly surprising that NO could be formulated as lipid emulsion for intravenous administration, and that this formulation made it possible for the NO to reach the lungs. Experiments indicate that an infusion already of 0.1 to 0.5 ml / kg body weight results in minor, but significant increases in expired NO, in animals given L-NAME to inhibit the endogenous NO production.

The results also indicated that the infusion of NO in lipid emulsion protected the animals against the lethal effects of pulmonary embolism in NO-synthesis inhibited animals. It is contemplated that the NO infusion excerts vasodialtory effects in the pulmonary circulation, and/or mild vasodialtory effect on systemic circulation, and inhibits thrombocyte aggregation, or a combination of these effects.

Nitrates are presently used to treat the symptoms of angina (chest pain). Nitrates work by relaxing blood vessels and increasing the supply of blood and oxygen to the heart while reducing its work load. Examples of presently available nitrate drugs include:

Nitroglycerin (glyceryl nitrate) (1,2,3-propantriol-nitrate), which is today mostly taken orally to curb an acute attack of angina. Strong headaches and dizzyness

due to the rapid and general vasodilatory effect are frequently encountered side-effects. Nitroglycerin infusion concentrates are also available, and diluted in glucose or physiological saline for intravenous infusion.

Isosorbide mononitrate (1,4:3,6-dianhydro-D-glucitol-5-nitrate), which is taken as prophylactic against angina pectoris. Tolerance develoment is a problem in long term treatment regimens. Frequent side-effects include headache and dizziness, as encountered with nitroglycerin.

Isosorbide dinitrate (1,4:3,6-dianhydro-D-glucitol-2,5-nitrate), which is taken both acutely and prophylactically against angina pectoris and cardiac insufficiency.

Pentaerythrityl nitrates, a group of organic nitrate, are known to exert long-term antioxidant and anti-atherogenic effects by as yet unidentified mechanisms. Pentaerythrityl tetranitrate has been investigated in the context of nitrate tolerance, an unwanted development in nitrate therapy, and experimentally tested in pulmonary hypertension.

Inorganic nitrates, such as potassium nitrate and sodium nitrate, have a long use as food preservatives. Nitrate has in general been considered to be potentially harmful, due to the theoretically possible formation of carcinogenic N-nitroso compounds in food, and in humans in vivo. Lately, the role of dietary nitrate has been reevaluated, in particular as the endogenous production of NO in the arginine-nitric oxide system and its role in host defence has been discovered.

L-arginine, and esters thereof, such as the ethyl-, methyl-and buthyl-L-arginine have been used to increase the endogenous production of NO.

Consequently, the present invention makes available a method for the treatment, alleviation or prevention of insufficient perfusion in an organ or organs in a human patient, wherein a compound, capable of delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO), is given intravenously to said patient. Insufficient perfusion includes states of insufficient perfusion of various etiology, in tissues or organs, including but not limited to transplanted tissues or organs.

According to an embodiment of the invention, said insufficient perfusion is insufficient perfusion of a section or sections of a lung, due to pulmonary embolism. Pulmonary embolism includes pulmonary embolism of various etiology, including but not limited to pulmonary thromboembolism and pulmonary gas embolism.

According to a preferred embodiment of the invention, said compound, capable of delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO), is chosen among gaseous NO, an inorganic nitrate, an organic nitrate, a pentaerytrityl nitrate compound, a isosorbide nitrate compound, nitroglycerin, L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine, or derivatives thereof.

According to a preferred embodiment, said compound is a compound capable of delivering NO, preferably gaseous NO and said injectable formulation is a lipid emulsion containing gaseous NO substantially in the absence of oxygen.

According to another embodiment, said compound is a compound capable of generating NO, preferably isosorbide mononitrate.

According to another embodiment, said compound is a compound capable of increasing the endogenous production of NO, preferably L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine.

The present invention also discloses the use of a compound capable delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO), for the manufacture of an injectable pharmaceutical formulation for the treatment of insufficient perfusion in an organ or organs in a human patient. Insufficient perfusion includes states of insufficient perfusion of various etiology, in tissues or organs, including but not limited to transplanted tissues or organs.

According to an embodiment of this use, said insufficient perfusion in an organ or organs is insufficient perfusion of a section or sections of a lung, due to pulmonary embolism. Pulmonary embolism includes pulmonary embolism of

various etiology, including but not limited to pulmonary thromboembolism and pulmonary gas embolism.

According to a preferred embodiment of this use, said compound is chosen among gaseous NO, a pentaerytrityl nitrate compound, a isosorbide nitrate compound, nitroglycerin, L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine, or derivatives thereof.

According to another embodiment, said compound is a compound capable of delivering NO, preferably gaseous NO and said injectable formulation is a lipid emulsion containing gaseous NO substantially in the absence of oxygen.

According to another embodiment, said compound is a compound capable of generating NO, preferably isosorbide mononitrate.

According to another embodiment, said compound is a compound capable of increasing the endogenous production of NO, preferably L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine.

The present invention also makes available a method for the manufacture of a NO-donating injectable composition, wherein a lipid emulsion, suitable for intravenous infusion, is de-oxygenised until substantially free from oxygen, and then purged with pure NO gas until a desired NO concentration is reached.

When preparing the NO emulsion according to the present invention, it is important that the medium is de-oxygenised before addition of NO. Otherwise the added NO will be decomposed by oxygen. It is also necessary to use, store and administer the emulsion under exclusion of oxygen. In practice, the storage vessels, vials, bottles or bags, as well as the tubes and cannulas should be non-peremable to oxygen. Except for this consideration, conventional apparatuses and practises for administering intravenous infusions can be used. Preferably an infusion pump is used.

Advantages

One important clinical advantage of the present invention is that the effects of the NO donating agent, when administered to the patient in the form of an infusion,

will be most significant in hypoxic tissue. When the NO donating agent, and in particular gaseous NO formulated in a lipid emulsion, is given as an intravenous infusion, the risk of so called proximal steal, i.e. increased blood flow in neighboring healthy vessels, is avoided. This is a common side effect of vasodilating substances, leading to lowered blood pressure and related systemic symptoms.

Further advantages will become evident to a skilled person upon study of the description and examples.

Examples

Methods

Anaesthesia and initial surgical procedures

The experiments were approved by the local animal ethics committee. Male white New Zealand rabbits (n=20, body weight 2.456±0.086 kg) were anaesthetised via an ear vein with sodium pentobarbital, 6 mg ml^{-1} in normal saline, 40-60 mg kg^{-1}. The animals were placed in supine position and tracheotomised just below the cricoid cartilage to allow mechanical ventilation using a tracheal cannula with an outer diameter of 5 mm. The animals were ventilated by a Harvard Apparatus rodent ventilator (model 683, Harvard Apparatus, South Natick, MA, USA). The ventilator was supplied with NO-free air using a charcoal filter (110 x 11 cm). Ventilation rate was 40 min^{-1} at constant volume where the tidal volume was initially adjusted to keep the end-tidal CO_2 at 4.5-5.3% as determined by a ventilatory monitor (Oscar-Oxy, Datex, Helsinki, Finland) sampling gas (150 ml min^{-1}, 15-20% of minute ventilation) from one of two side-arms connected to the tracheal cannula, and using a de-humidifying tube. The minute ventilation was 0.64-0.96 l min^{-1}. To the other side arm a pressure transducer (Statham, Hato Rey, Puerto Rico) was connected thus monitoring the insufflation pressure. The gas from the ventilator outlet was led through a switching valve to either of two beakers creating a positive end-expiratory pressure (PEEP) of 1-2 cmH_2O or 4-5 cmH_2O. During the experiment the gas flow was altered between the lower PEEP (9 min) and the higher PEEP (1 min) with an interval of totally 10 min. A

continuous infusion containing glucose (24.3 g l^{-1}), dextran 70 (26.5 g l^{-1}), $NaHCO_3$ (6,2 g l^{-1}), sodium pentobarbital (4.1 g l^{-1}) and pancuronium bromide (98 mg ml^{-1}) was administrated at a rate of 5 ml kg^{-1} h^{-1} via the same ear vein by means of a Terumo STC-521 syringe pump (Terumo Corp., Tokyo, Japan). A heparinized catheter was inserted in the left common carotid artery for blood pressure and heart rate recordings (pressure transducer, Staham, Hato Rey, Puerto Rico), and arterial blood sampling. Another catheter was inserted in the right jugular vein for drug and muscle emboli administration. Body temperature was maintained at 37-38.5°C by means of a heating pad connected to a thermostat. The muscles from the anterior compartment of the right lower hind limb were resected and placed in normal saline. Hereafter the animals were allowed a 30-60 min intervention-free period to obtain stable circulatory conditions and stable concentrations of expired NO.

NO measurements in exhaled air

NO concentration, in mixed exhaled gas, was continuously measured by means of a chemiluminescence based system (Niox, Aerocrine AB, Solna, Sweden) sampling at 100 ml min^{-1} at the end of a mixing chamber connected to the ventilator exhaust. The full mixing of expired air thus measured on was intermittently checked by monitor CO2 concentration in the same chamber. In a few experiments, gas for NO measurement was sampled from the trachea at the same point as for tidal CO2 measurements, thus yielding breath by breath NO concentrations. Calibration was done using certified NO standard gas in nitrogen (AGA Specialgas, Lidingö, Sweden).

Preparation of muscle emboli

The resected muscle tissue was cleared from all visible connective tissue and then homogenized and dissolved in normal saline to a concentration of 0,1 g muscle ml^{-1}. 50 IE heparin was added to the mixture. The homogenate was filtered through a filter (500µm) to prevent clotting in the three way stop-cock.

Experimental protocol

The animals were divided into two groups; 1) one group receiving a high dose (58 mg kg^{-1}) muscle homogenate and 2) a second group receiving the nitric oxide inhibitor L-NAME (30 mg kg^{-1}) 40 min before challenge with lower doses (30 to 7.5 mg kg^{-1}) muscle homogenate, since initial pilot experiments indicated a marked enhancement of emboli effects after L-NAME pretreatment. Blood samples were collected and analyzed for blood gases and acid-base status (ABL 300, Radiometer A/S, Copenhagen, Denmark) before L-NAME administration (group 2, time= -40 min) and shortly before muscle emboli challenge (group 1 and 2, time=-5 min). The muscle homogenate was infused by means of an infusion pump (CMA/100, Microinjection Pump, Carnegie Medicine AB, Stockholm, Sweden) with a flow of 150 µl kg^{-1} min^{-1} via a three way stop-cock into a carrier flow (864 Syringe Pump, Univentor LTD., Zejtun, Malta) of 150 µl kg^{-1} min^{-1} normal saline through the jugular vein catheter until full muscle emboli dose for each group was received. Arterial blood samples were collected and analysed at 10 min, 20 min, 40 min and 60 min after embolisation. NO concentration in exhaled gas, end-tidal CO_2, heart rate, mean arterial pressure and insufflation pressure was continuously monitored on a Grass Polygraph (Grass Instruments Co, Quincy, Mass, USA) during the experiments.

Experiment on intravenous infusion of NO dissolved in lipid emulsion

One rabbit pre-treated with L-NAME (30 mg kg^{-1}) received one infusion with NO-gas dissolved in normal saline and one with NO-gas dissolved in lipid emulsion through the catheter in the jugularis vein without carrier flow. The infusion rate was for both liquids 0.5 ml kg^{-1} min^{-1}. There was a recovery period between the two infusions for about 200 min.

The infusion liquids were created the same way. First the liquid was de-oxygenated for 20 min, in a gas-close glass chamber with a rubber membrane with an inert gas; in this case helium gas, but nitrogen, argon etc could also be used. After this no oxygen were allowed to enter the liquid throughout the following procedure. The liquid was then purged with pure NO for a few minutes.

The liquid was then collected through the rubber membrane in a gas-close syringe with needle and from this syringe infused by means of syringe pump (864 Syringe Pump, Univentor LTD., Zejtun, Malta) in the jugular vein catheter.

Drugs

Heparin was purchased from Kabi Vitrum, Stcokholm, Sweden, pancuronium bromide (Pavulon®) was from Organon, Oss, Holland, sodium pentobarbital was from Apoteksbolaget, Stockholm, Sweden and dextran 70 (Macrodex®) was from Pharmalink, Spånga, Sweden. L-NAME (N^G-nitro-L-arginine methyl ester) and routine chemicals were purchased from Sigma Chemical Company, St Louis, Missouri, USA.

Statistics

Statistical data are given as mean and standard error of the mean (SEM). Statistical significance was calculated by means of repeated measurements ANOVA on ranks with Dunnet's post hoc analysis. $P<0.05$ was assigned as significance difference. All statistical calculations were done by using a computer program (SigmaStat, Jandel, San Rafael, CA, USA).

Results in experiments on intravenous infusion of NO dissolved in liquid medium

Effects of L-NAME-infusion

Infusion of L-NAME (30 mg kg^{-1}) throughout 10 min decreased exhaled nitric oxide (from 19 ppb to <1 ppb, figure 1), increased systemic mean arterial blood pressure (MAP, from 103 cmH_2O to 128 cmH_2O), and lowered heart rate (HR, from 274 beats min^{-1} to 258 beats min^{-1}). End-tidal CO_2 and the relevant blood gas parameters were normal.

Infusion of NO-gas dissolved in normal saline

Rapid infusion (0.5 ml kg^{-1} min^{-1} during 30 min) of NO-gas dissolved in normal saline decreased MAP (from 128 cmH_2O to 75 cmH_2O), increased HR (from 258 beats min^{-1} to 295 beats min^{-1}), and slightly increased exhaled nitric oxide (from 0 ppb to 2.5 ppb, figure 1). The metHb fraction increased dramatically (from 0.1 % to 20 %, figure 2). About 200 min after the infusion, the animal had almost

completely recovered, and exhaled NO, MAP, HR and metHb were 0.8 ppb, 123 cmH_2O, 313 beats min^{-1} and 2.5 % respectively.

Infusion of NO-gas dissolved in lipid emulsion

Upon infusion (0.5 ml kg^{-1} min^{-1} during 30 min) of NO-gas dissolved in lipid emulsion exhaled NO increased (from 0.8 ppb to 32.5 ppb, figure 1), MAP fell from 106 cmH_2O to 55 cmH_2O while HR and metHb fraction (figure 2) were hardly affected.

Discussion on intravenous infusion of NO dissolved in liquid medium

The results clearly show that the administration of NO, via the blood circulation to the lungs, dissolved in a liquid medium, is heavily increased (about 15 times), monitored as exhaled NO, when NO is dissolved in lipid emulsion compared to normal saline. The present inventors point out a noticeable disadvantage of dissolving NO in normal saline compared to lipid emulsion, in that metHb is greatly increased. Generation of metHb may be serious if arterial oxygen saturation is reduced, for example in conditions with pulmonary hypertension like pulmonary embolism. In this experiment, the present inventors used a very high infusion rate and therefore MAP decreased significantly, but believe that a much slower infusion rate is sufficient to generate beneficial effects in the lung in conditions with pulmonary hypertension or tromboembolism and may achieve this without causing a major decrease in the systemic arterial blood pressure. Notice that exhaled NO increased to 32.5 ppb from <1 ppb and that normal levels of NO in mixed exhaled breath is approximately 20 ppb. Further it might not be necessary to generate these levels in exhaled gas as the beneficial effects probably are on the vasculature. The magnitude of the fall in MAP could also partly be due to the inhibition of endogenous NO generation.

In another experiment, the present inventors successfully treated venous gas embolism with NO dissolved in lipid emulsion, in an animal with inhibited NO production, whereas it was impossible to treat the same condition with inhaled nitric oxide in the same experimental setting. The beneficial effects may be induction of vasodilation in the pulmonary vasculature, inhibition of aggregation of

trombocytes and/or minor vasodilation in the whole or parts of the systemic circulation, for example in the coronary circulation.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

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Claims

1. A method for the treatment, alleviation or prevention of insufficient perfusion in an organ or organs in a human patient, **characterized in** that a compound, capable of delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO), is given intravenously to said patient.

2. The method according to claim 1, wherein said insufficient perfusion is insufficient perfusion of a section or sections of a lung, due to pulmonary embolism.

3. The method according claim 1 or 2, wherein said compound is chosen among gaseous NO, an inorganic nitrate, an organic nitrate, a pentaerytrityl nitrate compound, a isosorbide nitrate compound, nitroglycerin, L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine, or derivatives thereof.

4. The method according to claim 3, wherein said compound is gaseous NO and said injectable formulation is a lipid emulsion containing gaseous NO substantially in the absence of oxygen.

5. The method according to claim 3, wherein said compound is isosorbide mononitrate or a derivative thereof.

6. The method according to claim 3, wherein said compound is L-arginine, or a derivative thereof.

7. Use of a compound capable delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO), for the manufacture of an injectable pharmaceutical formulation for the treatment of insufficient perfusion in an organ or organs in a human patient.

8. The use according to claim 7, wherein said insufficient perfusion in an organ or organs is insufficient perfusion of a section or sections of a lung, due to pulmonary embolism.

9. The use according to claim 7 or 8, wherein said compound is chosen among gaseous NO, a pentaerytrityl nitrate compound, a isosorbide nitrate compound,

nitroglycerin, L-arginine, an L-arginine ester such as ethyl-, buthyl- or methyl-L-arginine, or derivatives thereof.

10. The use according to claim 9, wherein said compound is gaseous NO and said injectable formulation is a lipid emulsion containing gaseous NO substantially in the absence of oxygen.

11. The use according to claim 9, wherein said compound is isosorbide mononitrate.

12. The manufacture of a NO-donating injectable composition, characterized in that a lipid emulsion, suitable for intravenous infusion, is de-oxygenised until substantially free from oxygen, and then purged with pure NO gas until reaching a desired concentration of NO in the emulsion.

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Abstract

Insufficient perfusion can be treated, alleviated or prevented through the intravenous administration of a compound, capable of delivering, or generating nitric oxide (NO), or capable of increasing the production of endogenous nitric oxide (NO).

(Fig. 10)

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Fig. 1

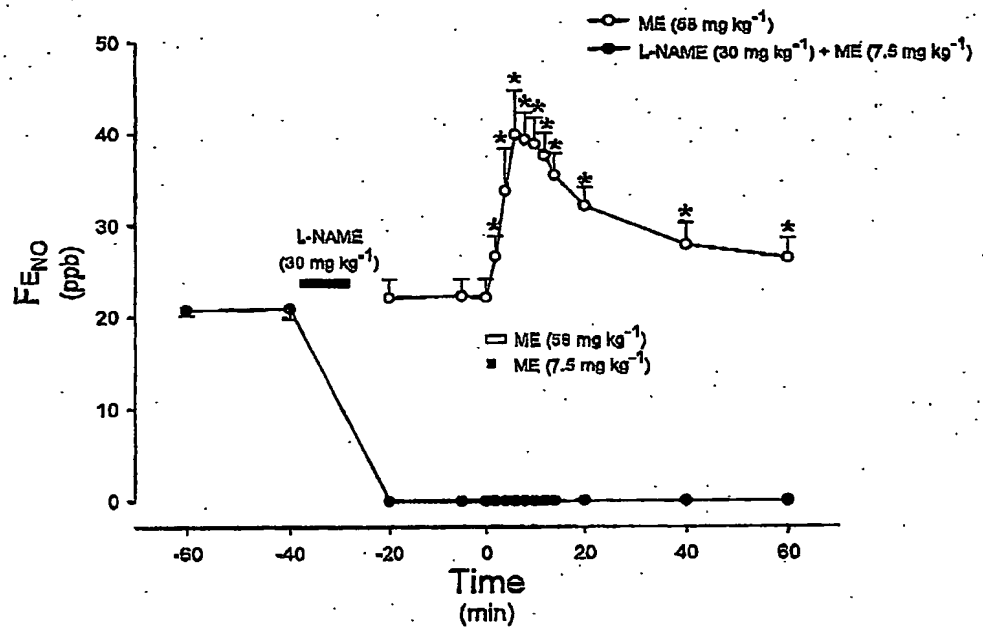


Fig. 2

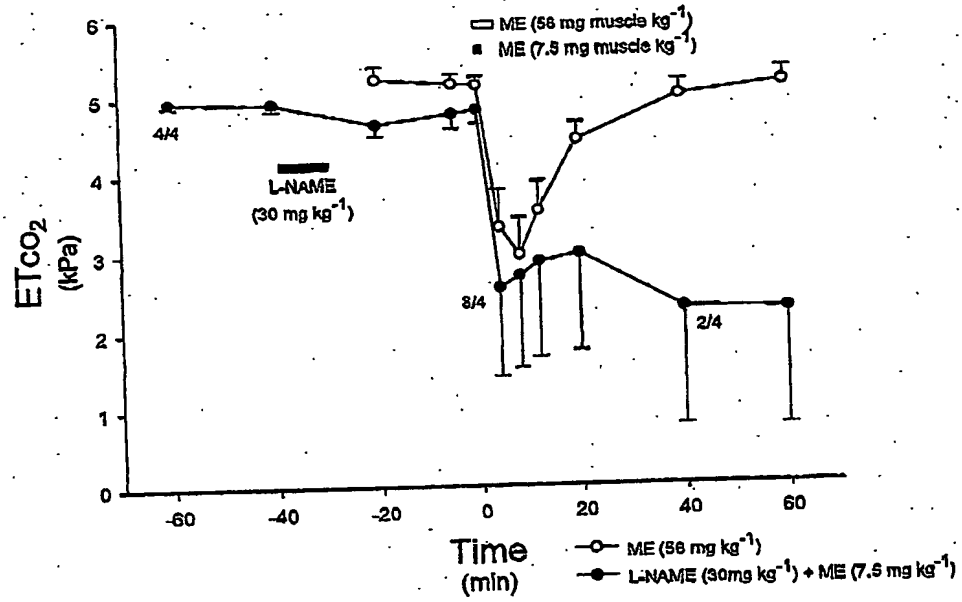

ME (56 mg muscle kg^{-1})
ME (7.5 mg muscle kg^{-1})
4/4
L-NAME
(30 mg kg^{-1})
8/4
2/4
$ETCO_2$ (kPa)
Time (min)
ME (56 mg kg^{-1})
L-NAME (30mg kg^{-1}) + ME (7.5 mg kg^{-1})

Fig. 3

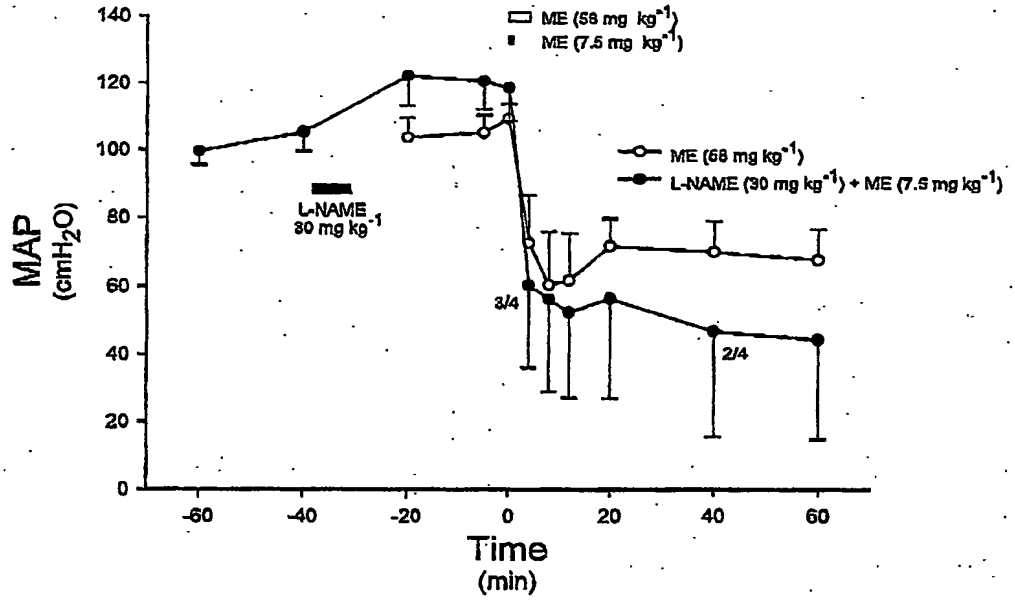

ME (58 mg kg-1)
ME (7.5 mg kg-1)
ME (58 mg kg-1)
L-NAME (30 mg kg-1) + ME (7.5 mg kg-1)
L-NAME 30 mg kg-1
MAP (cmH2O)
3/4
2/4
Time (min)

Fig. 4

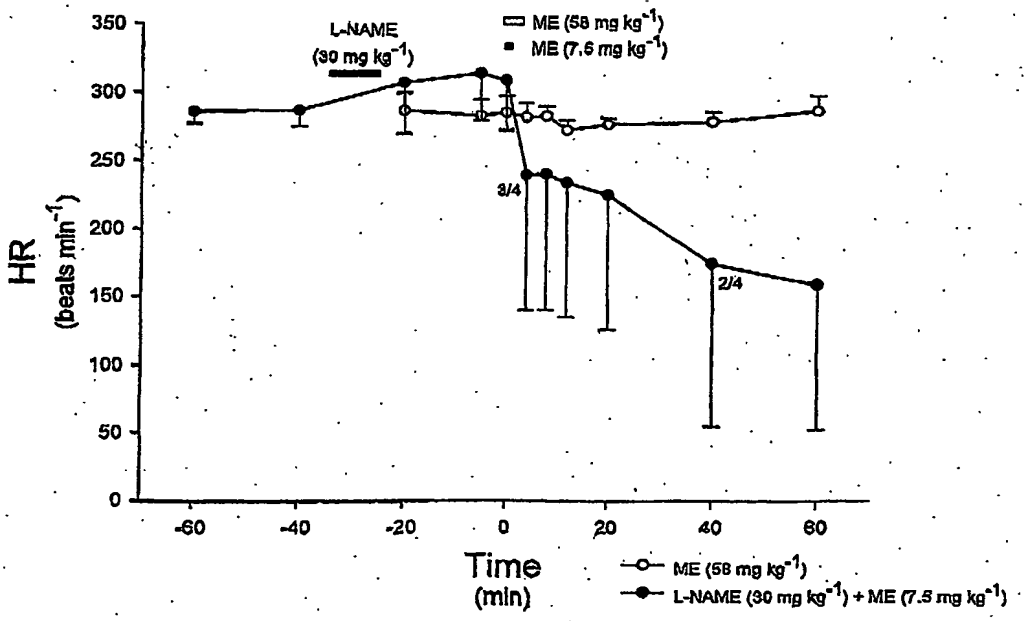

L-NAME (30 mg kg-1)
ME (58 mg kg-1)
ME (7.5 mg kg-1)
HR (beats min-1)
3/4
2/4
Time (min)
ME (58 mg kg-1)
L-NAME (30 mg kg-1) + ME (7.5 mg kg-1)

Fig. 5

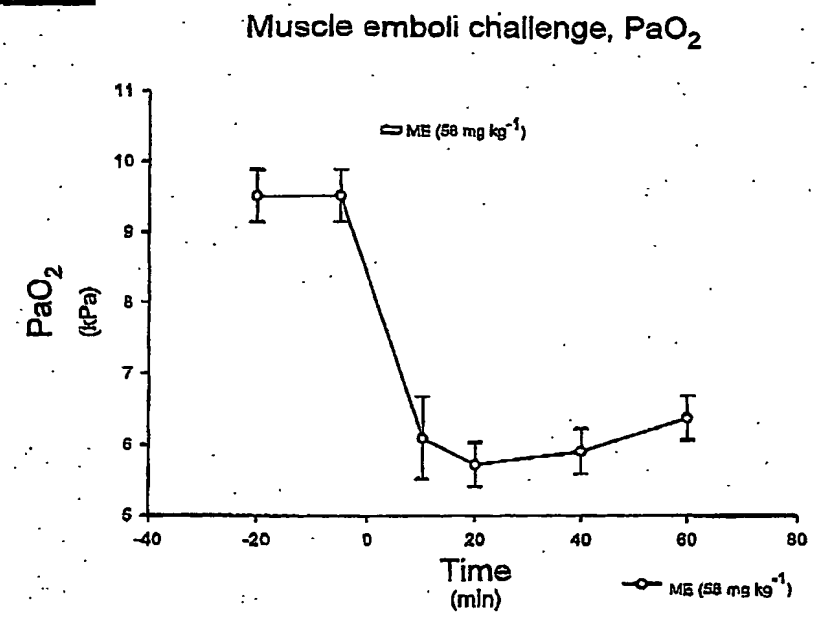
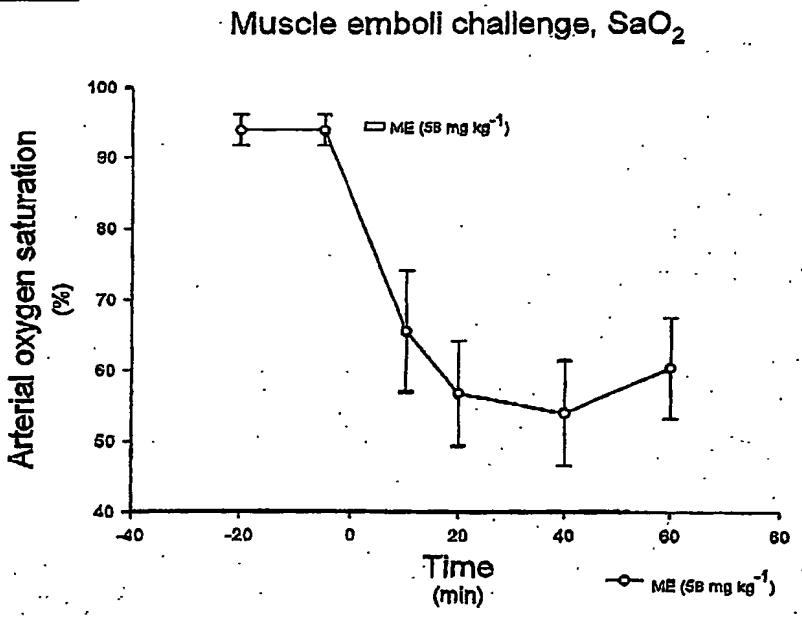


Fig. 6



5

Fig. 7

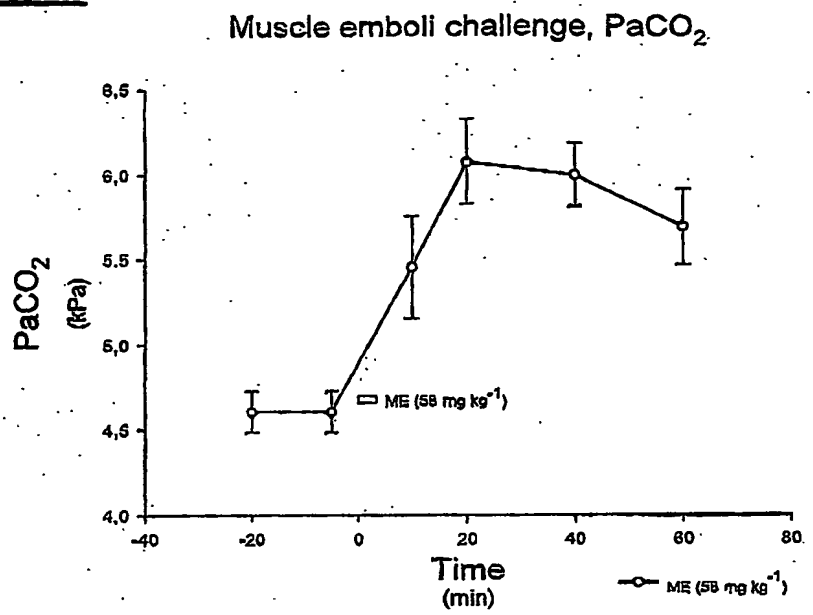
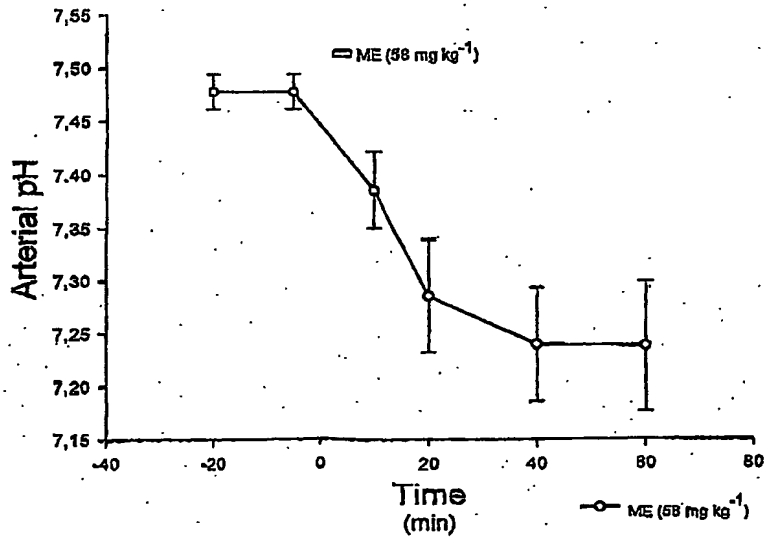
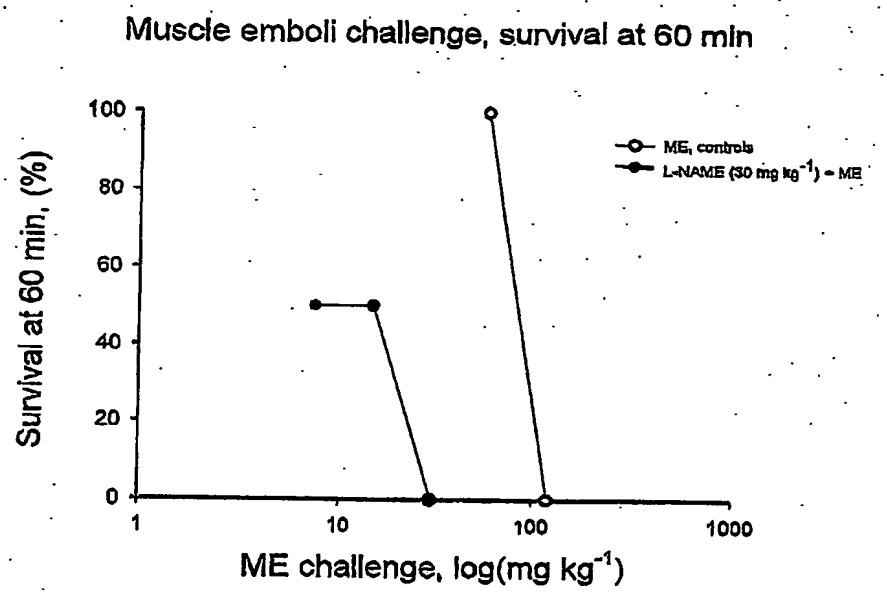

Muscle emboli challenge, $PaCO_2$
$PaCO_2$ (kPa)
6,5
6,0
5,5
5,0
4,5
4,0
ME (58 mg kg^{-1})
-40
-20
0
20
40
60
80
Time (min)
ME (58 mg kg^{-1})

Fig. 8


Muscle emboli challenge, arterial pH
Arterial pH
7,55
7,50
7,45
7,40
7,35
7,30
7,25
7,20
7,15
ME (58 mg kg^{-1})
-40
-20
0
20
40
60
80
Time (min)
ME (58 mg kg^{-1})

5

Fig. 9



survival

Fig. 10

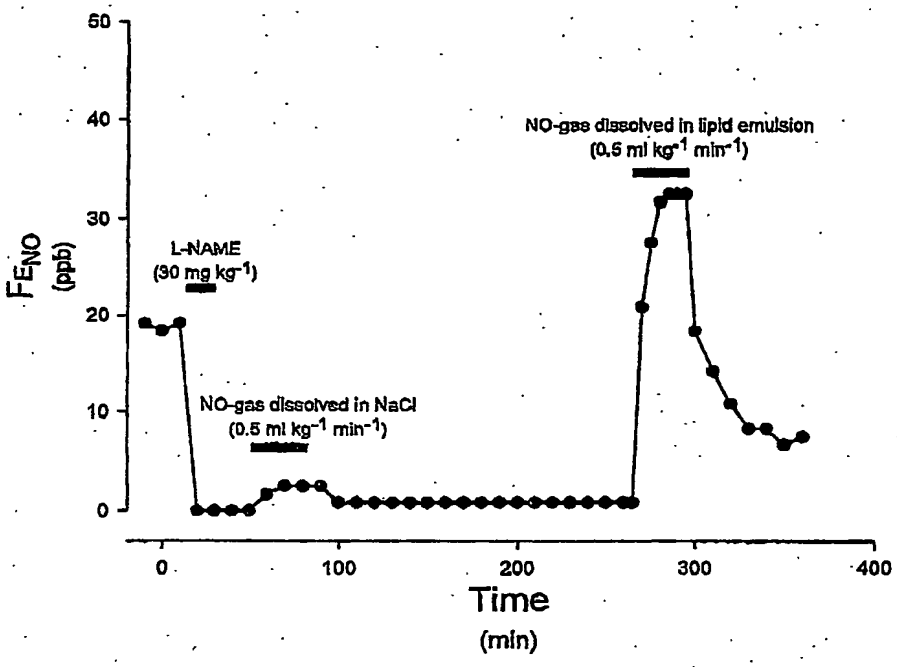
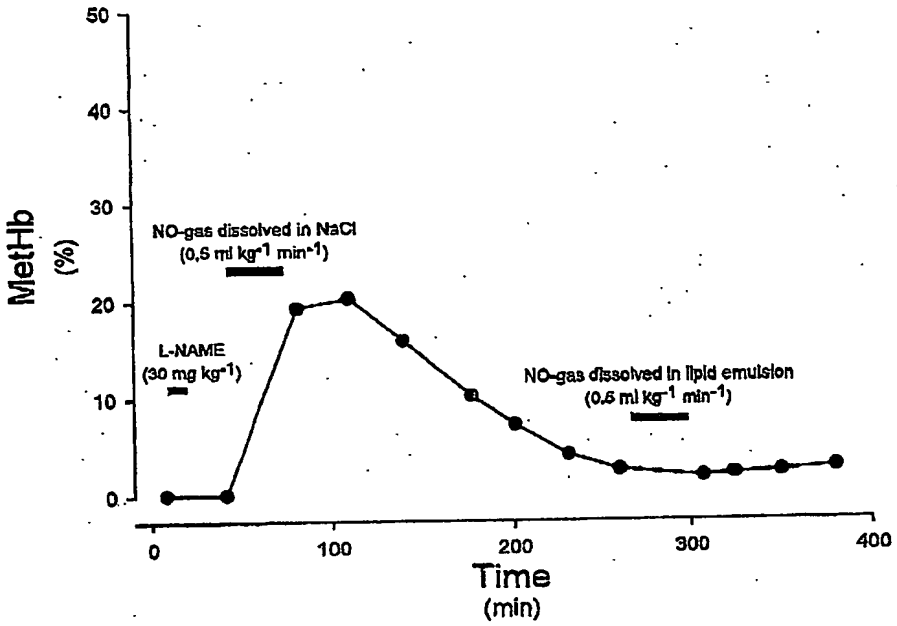


Fig. 11



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